

# **Standard Operating Procedure for Isolation, Extraction and Analysis of Atrazine, DEA and DIA**

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## **1.0 General Principal**

Atrazine, DEA and DIA will be isolated from Lake Michigan water samples using Carbowpak SPE cartridges. Analytes will be extracted from SPE cartridges with 7 mL of 85% dichloromethane (DCM), 15% methanol (MeOH) solution (vol:vol), followed by 2 mL of MeOH. Concentrated extracts will be spiked with internal standard and analyzed by gas chromatography-mass spectrometry.

## **2.0 Isolation**

Samples will be collected on board the EPA research vessel Lake Guardian according to the Standard Operating Procedure for sample collection. Samples will be transported to the University of Minnesota and stored at 4°C until processing. Samples will be stored for less than 30 days until extraction.

Carbowpak (Supelco) solid phase extraction cartridges (SPE) will be used for herbicide isolation. SPE cartridges (250 mg) will be cleaned prior to use with 3 mL of 85% DCM, 15% MeOH solution (vol:vol). Just prior to isolation cartridges will be conditioned ~ 2 mL of MeOH, followed by ~2 mL of Milli Q water. Conditioning the cartridge is critical to achieving complete herbicide isolation, and facilitating the passing of sample water. Conditioning of SPE cartridges should be done less than 15 minutes before passing sample water through the cartridge.

Solvents and water are pulled through the SPE cartridge under vacuum. Samples are interfaced to the SPE cartridge using Teflon tubing and a rubber stopper. One end of the Teflon tubing is inserted through the stopper about one inch. The stopper and protruding tubing are then pressed into the reservoir end of the SPE cartridge. The other end of the Teflon tubing is placed in the sample bottle. The lower end of the extraction cartridge is connected to vacuum, and sample water is pulled through the cartridge at ~ 20 mL/min. Flow rate is controlled by adjusting the vacuum.

Teflon tubing is cleaned before each extraction by placing one end of the tubing into a reservoir of MeOH, and inserting the stopper end into a “dummy” extraction cartridge as though it were a sample. MeOH is then pulled through the tubing under the vacuum. This procedure is repeated with Milli-Q water as a rinse.

Prior to isolation the sample will be spiked with 50 µL of a surrogate solution containing terbutylazine. The sample should be spiked on the same day it is to be passed through the SPE cartridge. A 50 µL micro-pipetter fitted with a glass capillary tube is used for surrogate spiking. The same micro-pipetter should be used for spiking all samples.

Sample volumes are determined using an E2000 Mettler top loading balance. The full sample bottle is placed on the balance and the balance is tared to zero. After the sample has been passed through the SPE cartridge the empty sample bottle is re-weighed and the sample volume calculated by difference.



Once the sample has been passed through the SPE cartridge, the cartridge is labeled, disconnected from the vacuum line, wrapped in aluminum foil, and stored at 4°C. Cartridges are labeled with all information on the sample bottle including: Lake, station, date, depth and code number. The volume of the sample is also put on the label. All extraction information is also entered into the lab log book. The lab log book should contain all information transferred from the field sampling log book, along with the data of sample isolation procedure, volume of sample, and amount of surrogate added.

### 3.0 Extraction

SPE cartridges are removed from cold storage, the aluminum foil removed, and allowed to reach room temperature.

Herbicides are extracted from the SPE cartridge by passing 7 mL of 85% DCM, 15% MeOH (vol:vol) solution, followed by 2 mL of MeOH through the cartridge. Extraction solvent pass through the cartridge by gravity. The small amount of solvent remaining in the cartridge after extraction is forced through using a syringe fitted with a rubber stopper. The bored out stopper is inserted into the reservoir end of the cartridge and remaining solvent is forced through using the syringe.

As the extraction solvent moves through the SPE cartridge, a small spatula full of anhydrous sodium (0.75 g) sulfate is added to the extractant in the centrifuge tube. The sodium sulfate removes any water remaining from sample isolation. The solvent is then collected by the centrifuge tube. Care must be taken to insure that the solvent flows evenly through each step of the extraction, and does not build up and overflow the pipette. Care must also be taken to insure that the leir end of the cartridge is centered in the pipette, if it is not solvent may drip along the outsides of the pipette.

Once all the extraction solvent has been collected in the centrifuge tube, the tube is labeled with all information on the SPE cartridge, sealed with aluminum foil, lined, capped, and stored at 4°C. If any solvent is lost, or spilled during extraction this should be noted on the label, and in the lab log book.

Extracted samples should be stored no longer than two weeks in centrifuge tubes. In less than two weeks the extracted sample should be solvent reduced and concentrated. Sample volume is reduced by placing the centrifuge tube in a Supelco Visidry Evaporation Manifold, attached to zero graded nitrogen gas source. Solvent is evaporated under a slow steady stream of nitrogen. About 25 pounds of pressure is desirable, but all samples should be visually inspected to insure that solvent is not mixing violently or splashing.

Samples should be blown down to 0.3 mL in the centrifuge tube. When the sample has been reduced to 0.3 mL it should be removed from the evaporation manifold, capped and placed in a test tube rack. The sample should then be transferred immediately to a 2 mL amber vial. The sample should not be stored in the reduced volume condition as it may evaporated to dryness. The sample should be transferred to a 2 mL vial using a 9 inch disposable pipette and pipette bulb. The centrifuge tube should be rinsed with 85% DCM: 15% MeOH solution three times, and the rinse also transferred to the amber vial.



The amber vial should be labeled with all information on the centrifuge tube, and stored at 4°C. If any solvent is spilled or lost during transfer this should be noted on the label and in the lab log book.

## 4.0 Analysis

Approximately 15 samples at a time should be prepared for analysis. Amber vials should be removed from cold storage and allowed to reach room temperature before uncapping. It is important that samples be allowed to reach room temperature before uncapping, otherwise condensation forming on the vials may enter the sample, and contaminated the sample with water.

Once the samples have reached room temperature they can be uncapped and placed in the Supelco Visidry Evaporation Manifold. The 2 mL vials should be concentrated to ~150 µL under a slow steady stream of nitrogen.

After the samples have been concentrated they should be spiked with 2 µL of the internal standard solution containing deuterated, d<sub>5</sub>ethyl atrazine and 4,4'-dibromobiphenyl. Samples should be spiked using a 2 µL Hamilton syringe fitted with a cheney syringe guide. The cheney syringe guide assures that every injection spike is identical. The cheney adapter has a stopper fitted on the guide so that each injection is automatically set to deliver 2 µL. The stopper guide is calibrated at the beginning of the project to properly set the syringe for exactly a 2 µL injection. This is accomplished by making repeated injections of 20°C water onto an analytical balance, and adjusting the guide stop until the syringe consistently deliver 2 µL. It is very important the internal standard solution spikes are accurate and consistent since quantitation is based on these injection volumes (or mass per injection volume). Once a sample has been spiked with internal standard, the date and amount should be recorded in the lab log book.

Samples that have been spiked with internal standard should be run within one week after spiking. As long as samples are stored at 4°C, and in amber vials the internal standards are stable, but prompt analysis of the samples is recommended to lessen the chance of errors, sample evaporation, breakage, loss, and to enhance analytical consistency.

When a set of samples has been spiked and is ready for analysis the following steps should be followed:

1. Remove spiked samples and calibration standards from cold storage, and allow to reach room temperature.
2. Initiate AutoTune calibration function of GC-ms *chem station*.
3. Transfer ~ 50 µL of each sample and each calibration standard to a 2 mL auto sampler vial with 250 µL micro vial insert.
4. Four calibration standards are run daily. Calibration standards are prepared from a stock solution, and are the same calibration standards used by Schottler and Eisenreich.
5. Load samples and calibration standards into auto sampler tray, and log samples into GC-MS *chem station* sequence function.

6. Enter sample order, sample identification, and Adaption values in GC-MS sample run log book.
7. Fill auto sampler solvent wash vials with DCM and MeOH.
8. Run calibration standard (1) twice, and inspect to make sure all peaks are present and peak shape is good.
9. Initiate auto sampler and run *chem station* sequence function.
10. Acquire data and use internal standard method to quantitate, see Section 10.0 of QAPjP.

## **5.0 Blanks**

### **5.1 Procedural Blanks**

One procedural blank should be run with every 20-25 samples.

A procedural blank is a SPE cartridge that is processed identical to a sample with the exception of water being passed through the cartridge. A procedural blank is cleaned, eluted, concentrated and analyzed identical to a sample.

### **5.2 Solvent Blanks**

Once every six months, or any time a procedural or field blank shows contamination a solvent blank should be run.

A solvent blank is simply the analytical reagents analyzed by GC-MSD for possible contaminants.

If a solvent blank shows contamination the auto sampler injection syringe should be changed and second solvent blank run. Blank contamination often results from dirty syringe needles, and should be tested as described to eliminate or confirm solvent as contaminated.

## **6.0 Sample Locations**

*Remember:*

Rinse three times  
Fill two bottles per one sample  
Label bottle and cap

### **6.1 Open Water Stations:**

#### **6.1.1 If Stratified**

\*Mid Epi

\*Mid Hypo (If possible, sample hypo at depth that corresponds to mean particle mass)

as measured by transmissometry)

6.1.2 If Not Stratified

\*2 ft. below surface

\*Mid-water column

Collect Duplicates of two open water stations. One station in Northern LM and one in Southern LM. Put “DUP” on label.

6.2 Master Stations:

6.2.1 If Stratified

Stations 18 and 41

\*2 ft below surface plus duplicate

\*5 ft below surface

\*Mid Epi

\*Thermo

\*Mid Hypo

\*5 ft off bottom plus duplicate

\*plus duplicates of all depths at St. 23 and put “BE DUP” on label

6.2.2 If Not Stratified

All Master Stations: (18,23,27,41,47)

\*2 ft below surface

\*Mid water column

\*5 ft off bottom

\*Duplicates of all depths at Stations 18,23,41

(Put BE on St. 23 label)

Mark St. 18 and St. 41 Duplicates with “DUP,” St. 23 with “BE DUP” in addition to regular sample label.